Appl. No. 09/714,040
Amendment dated April 30, 2007
Reply to Office Action of November 30, 2006

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This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1-24. (cancelled)

25. (currently amended) A composition comprising a monospecific $F(ab')_2$, wherein the $F(ab')_2$:

(a) is free of $F(ab')_2$ having hinge region intrachain disulfide bonds; and

(b) comprises a first and a second Fab', each first and second Fab' has a single hinge region cysteine residue and ~~comprising a CH1 domain fused to an amino acid sequence of up to 10 amino acids, wherein the amino acid sequence of up to 10 amino acids has~~ comprises a C terminal amino acid sequence of Cys-Ala-Ala, and wherein the cysteine of the first Fab' forms a bond with the cysteine of the second Fab' to form the monospecific $F(ab')_2$, and wherein the monospecific $F(ab')_2$ lacks glycosylation.

26-38. (cancelled)

39. (previously presented) The composition of claim 25, wherein the $F(ab')_2$ polypeptide lacks a heavy and light interchain disulfide bond.

40. (previously presented) A composition comprising a $F(ab')_2$ comprising a first and second Fab', wherein each first and second Fab' comprises a CH1 region fused to an amino acid sequence consisting of Cys-X-X, wherein one or both Xs are absent or X is Ala, Arg, Asp or Pro.

41. (previously amended) The composition of claim 40, wherein the amino acid sequence consists of Cys-Ala-Ala or Cys-Pro-Pro.

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42. (previously presented) The composition of claim 40, wherein the $F(ab')_2$ lacks a heavy and light interchain disulfide bond.

43. (previously presented) The composition of claim 25, wherein the $(Fab')_2$ lacks glycosylation.

44. (currently amended) A composition comprising a monospecific $F(ab')_2$ produced by the process of:

a) expressing a nucleic acid sequence encoding an inducible promoter operably linked to nucleic acid encoding a Fab' in a microbial host cell under conditions suitable for secretion of the Fab' to the periplasmic space; wherein the Fab' has a single hinge region cysteine residue and ~~comprises a CH1 domain fused at its C-terminus to an amino acid sequence of up to 10 amino acids, wherein the amino acid sequence of up to 10 amino acids~~ comprises a C terminal amino acid sequence of Cys-Ala-Ala;

b) recovering the Fab' from the host cell and coupling the free thiol of each Fab' to form a the monospecific $F(ab')_2$.

45-48. (cancelled)

49. (currently amended) A composition comprising a Fab' coupled to a heterologous molecule produced by the process of:

a) expressing a nucleic acid sequence encoding an inducible promoter operably linked to a nucleic acid encoding a Fab' in a microbial host cell under conditions suitable for secretion of the Fab' to the periplasmic space; wherein the Fab' has a single hinge region cysteine residue and ~~comprises a CH1 domain fused at its C terminus to an amino acid sequence of up to 10 amino acids, wherein the amino acid sequence~~ comprises a C terminal amino acid sequence of Cys-Ala-Ala;

b) recovering the Fab' from the host cell and coupling the free thiol of the Fab' with the heterologous molecule.

50. (previously presented) The composition of claim 49, wherein the heterologous molecule is a detectable label, or solid support.

51. (previously presented) The composition of claim 50, wherein the detectable label is a radionuclide or fluorescent probe.

52. (cancelled)

53. (cancelled)

54. (currently amended) A composition comprising ~~an antibody fragment~~ a Fab' fragment coupled to a heterologous molecule produced by the process of:

a) expressing a nucleic acid ~~sequencer~~ under the control of an inducible promoter, ~~encoding the antibody~~ wherein the nucleic acid encodes a light chain variable domain, a heavy chain variable domain and a CH1 domain fused to one or more cysteines or a cysteine-containing polypeptide of about 1-10 amino acid residues in place of an immunoglobulin hinge region ~~fragment~~ in a microbial host cell under conditions suitable for secretion of the antibody fragment to the periplasmic space, ~~; wherein the antibody fragment is a Fab' in which a heavy chain CH1 domain is fused to one or more cysteines or a short cysteine-containing polypeptide of about 1-10 residues~~ and wherein the ~~Fab' comprises a C terminal amino acid sequence of Cys-Ala-Ala~~ cysteine containing polypeptide has a single cysteine residue; and

b) recovering the Fab' ~~antibody fragment~~ from the host cell and coupling the free thiol of the Fab' with the heterologous molecule, and wherein the Fab' lacks glycosylation.

55. (previously presented) The composition of claim 54, wherein the heterologous molecule is a detectable label, or solid support.

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56. (previously presented) The composition of claim 55, wherein the detectable label is a radionuclide or fluorescent probe.

57. (previously presented) The composition of claim 54, wherein the short cysteine containing polypeptide comprises a part of a hinge region.

58. (currently amended) The composition of claim 57, wherein the part of the hinge region has all of the hinge region cysteines C terminal to the first cysteine deleted ~~or substituted~~.

59. (previously presented) The composition of claim 54, wherein the Fab' lacks glycosylation.

60. (currently amended) A composition comprising a monospecific $F(ab')_2$ produced by the process of:

a) expressing a nucleic acid ~~sequence encoding~~ under control of an inducible promoter, wherein the nucleic acid encodes a Fab' in a microbial host cell under conditions suitable for secretion of the Fab' to the periplasmic space; wherein the ~~antibody fragment is a~~ Fab' ~~in which~~ comprises a heavy chain CH1 domain ~~is~~ fused to one or more cysteines or a ~~short~~ cysteine-containing polypeptide of about 1-10 residues in place of an immunoglobulin hinge region, and wherein the ~~Fab' comprises a C terminal amino acid sequence of Cys-Ala-Ala~~ cysteine containing polypeptide has a single cysteine residue;

b) recovering the Fab' from the host cell and coupling the free thiol of each Fab' to form a the monospecific $F(ab')_2$,and wherein the monospecific $F(ab')_2$ lacks glycosylation.

61. (previously presented) The composition of claim 60, wherein the heterologous molecule is a detectable label, or solid support.

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62. (previously presented) The composition of claim 61, wherein the detectable label is a radionuclide or fluorescent probe.

63. (previously presented) The composition of claim 60, wherein the short cysteine containing polypeptide comprises a part of a hinge region.

64. (currently amended) The composition of claim 63, wherein the part of the hinge region has all of the hinge region cysteines C terminal to the first cysteine deleted ~~or substituted.~~

65. (previously presented) The composition of claim 60, wherein the Fab' lacks glycosylation.

66. (new) The composition of claim 54, wherein the Fab' comprises a C terminal amino acid sequence Cys-Ala-Ala.

67. (new) The composition of claim 60, wherein the Fab' comprises a C terminal amino acid sequence Cys-Ala-Ala.

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